

Effect of carbamide peroxide bleaching agents on the physical properties and chemical composition of enamel

HALE CIMILLI, DDS, PHD & CORNELIS H. PAMEIJER, DMD, DSC, PHD

ABSTRACT: ***Purpose:*** To evaluate the effect of two commercially available carbamide peroxides bleaching agents, Opalescence and Nite-White, in concentrations of 10, 15 and 16%, on enamel. ***Materials and Methods:*** Vickers hardness measurements (VH) were made at the surface and 110 μm below the surface on human enamel samples that were treated for 5 or 10 days, 6 hrs/day. Furthermore, infrared spectrophotometry (IR), Fourier transform infrared spectrophotometer (FTIR) and X-ray diffraction (XRD) measurements were performed after the VH measurements were completed. ***Results:*** Using a one-way ANOVA, statistically significant differences in VH could be established between surface and subsurface VH values. For all groups, including the control group, the surface VH values were statistically significantly higher than subsurface VH. IR, FTIR and XRD established a change from hydroxyapatite to primary calcium ortho phosphate $[\text{Ca}(\text{H}_2\text{PO}_4)_2]$ for all experimental groups except for 10% Opalescence tested for 5 or 10 days. (*Am J Dent* 2001;14:63-66).

CLINICAL SIGNIFICANCE: Some bleaching formula concentrations that were tested lowered the hardness of surface enamel significantly and caused dissolution of Ca^{2+} from enamel through conversion of hydroxyapatite. Nite-White in particular exhibited a reduction in VH and a change in hydroxyapatite. At this time the significance of these chemical changes in clinical practice is not known.

CORRESPONDENCE: Dr. Hale Cimilli, Zincirlikuyu Akademiler Sitesi 4/823 80600 Levent, Istanbul, Turkey. Fax: 90 212 66143 37. E-mail: halecimilli@superonline.com

Introduction

Currently there is a widespread use of bleaching agents to whiten teeth for esthetic purposes. The technique is easy and patient acceptance is high. The demands of the population today concerning their appearance are much higher than previously. Not only do teeth have to be aligned properly, they must be white as well. The night guard vital bleaching technique with a build-in reservoir offers a conservative, safe and cost effective method for bleaching teeth.¹ Studies have reported the effect of bleaching agents on enamel. With respect to the hardness of enamel, studies²⁻⁴ noticed no change in Knoop hardness of human enamel *in vitro* after exposure to 10% carbamide peroxide under a variety of conditions.

McCracken & Haywood⁵ reported no significant adverse effect of bleaching agents on subsurface levels of enamel. Haywood *et al.*^{6,7} using scanning electron microscopy to examine surface morphology of human enamel, also found no changes. However, Shannon *et al.*² demonstrated minor morphological changes on enamel surfaces treated with 10% carbamide peroxide solutions. The mechanism of action of bleaching agents is attributed to the oxidation of enamel and dentin molecules causing changes in color in the dentin.⁸⁻¹¹ Bleaching agents can cause loss of calcium and changes in the chemical composition of dental hard tissues.¹²⁻¹⁴

In spite of its widespread use, there is no general agreement as to the effect of bleaching agents on enamel. This study evaluated the physical properties and chemical composition of enamel following the use of different concentrations of two bleaching agents. The two entities used were conducted separately, one examined the hardness of the samples after treatment, the other analyzed the crystalline structure. Since the techniques that were used had completely different objectives, no correlations between the methods were made.

Materials and Methods

Recently extracted maxillary bicuspid, removed for orthodontic reasons, were used for this study. All teeth were examined at $\times 15$ for micro-cracks and surface defects using a dissecting stereomicroscope.³ The crowns of 45 teeth were cut at the CE-junction using a diamond saw^b under copious water cooling. Next, the crowns were cut in half in mesio-distal direction, and each section was further divided in a buccolingual direction resulting in four sections measuring approximately $5 \times 4 \times 2$ mm. Each section, with the enamel surface facing up, was mounted in an acrylic resin ring with autopolymerizing resin.

Therefore the total number of samples was 180. One section of each tooth served as control ($n=45$); three sections of each tooth were used for the bleaching agents ($3 \times 45 = 135$). For each group 15 samples were tested. Ten were used for VH tests and the remaining five for infrared absorption spectrophotometry^c (IR) (Model DMAX 1000), Fourier transform infrared spectrophotometry^d (FTIR) (Model PU9714) and X-ray diffractometry^e (XRD) (Model Mattison 1000). The aim of using three spectral analyses was to lend support to each individual technique in order to reach a more reliable and definite conclusion. IR spectrophotometry established a qualitative change in chemical composition, while FTIR determined quantitative changes. Powdered XRD was used to determine whether deviations in crystalline structure had taken place while in addition this technique supported the findings from IR and FTIR spectrophotometry. When X-rays of a certain wavelength and intensity strike the sample a portion is absorbed by the material while the remainder is scattered along the atomic plane within the sample according to Bragg's equation $n\lambda = 2d\sin\theta$. Each material exhibits a specific X-ray diffraction pattern with a

Table 1. Groups evaluated.

Group	Description
1	10% Opalescence, ^c applied for 6 hrs/day for 5 days (10%Op/5d/S*; 10%Op/5d/SS**)
2	10% Opalescence applied for 6 hrs/day for 10 days (10%Op/10d/S; 10%Op/10d/SS).
3	10% Nite-White Excell, ^d applied for 6 hrs/day for 5 days (10%NW/5d/S; 10%NW/5d/SS).
4	10% Nite-White Excell, applied for 6 hrs/day for 10 days (10%NW/10d/S; 10%NW/10d/SS).
5	16% Nite-White Excell, applied for 6 hrs/day for 5 days (16%NW/5d/S; 16%NW/5d/SS).
6	16% Nite-White Excell, applied for 6 hrs/day for 10 days (16%NW/10d/S; 16%NW/10d/SS).
7	15% Opalescence, applied for 6 hrs/day for 5 days (15%Op/5d/S; 15%Op/5d/SS).
8	15% Opalescence, applied for 6 hrs/day for 10 days (15%Op/10d/S; 15%Op/10d/SS).
9	Control enamel, stored in an incubator at 37°C for 5 days (Ctrl/5d/S; Ctrl/5d/SS).
10	Control enamel, stored in an incubator at 37°C for 10 days (Ctrl/10d/S; Ctrl/10d/SS).

* S = surface hardness. ** SS = Sub-surface hardness.

diagram that displays cps accumulations at specific angles (2 θ). Every peak in these diagrams represents "d" (d = interplanar distance between atoms), which corresponds to a specific atomic plane. XRD analysis¹⁵ resulted in the identification of crystalline powders, determination of lattice constants and demonstration of ionic substitutions. It distinguishes between crystalline and amorphous substances, determines crystal size and/or inhomogeneous strains. It establishes the preferred orientation of crystals and determines the positions of atoms in crystals. The following 10 groups were tested (Table 1).

All bleaching materials were applied per manufacturers' instructions. After 6 hrs of bleaching gel application, the samples were washed twice in distilled water and stored in an incubator at 37°C, wrapped in moist cotton, until the next application. Distilled water was used for storage of the samples rather than artificial saliva, to prevent the introduction of more variables. Hardness of the sample was tested using a Vickers hardness machine (Leitz Miniload^h model LL) with a diamond tip loaded with 50 g for 20 s. Measurements were made at three arbitrarily chosen locations. After completion of the surface hardness measurements, the samples were ground on wet silicone carbide paper from 600-1200 gritⁱ and $\pm 110 \mu\text{m}$ was removed from the surface. Progress of surface removal was continually monitored with a micrometer (Microfix^j 0.1, 0-25mm DIN863/I). After the desired amount of enamel had been removed another Vickers hardness measurement was done, this time with a 200 g load applied for 20 s. As a result of the sanding a larger surface was created and this time measurements could be made at five arbitrary locations. All measurements were tabulated and subjected to a statistical analysis using a one-way ANOVA at a 99% confidence level.

The 5 samples per group that were assigned for IR and FTIR spectrophotometry and XRD were carefully removed from the acrylic rings and ground to powder in an agate mortar.

Table 2. Mean and standard deviation of enamel surface Vickers hardness.

Group	Description	Mean \pm S.D.
1	10%Op/5d/S*	422.9 \pm 4.15
2	10%Op/10d/S	418.7 \pm 8.39
3	10%NW/5d/S**	423.6 \pm 3.86
4	10%NW/10d/S	419.1 \pm 7.32
5	16%NW/5d/S	415.1 \pm 2.13
6	16%NW/10d/S	408.4 \pm 3.95
7	15%Op/5d/S	422.6 \pm 5.52
8	15%Op/10d/S	416.7 \pm 7.06
9	Control/5d/S	439.2 \pm 5.57
10	Control/10d/S	437.1 \pm 9.69

* Op/5d/S = Opalescence treatment for 5 days, surface analysis. ** NW = Nite-White.

Table 3. Mean and standard deviation of enamel 110 μm sub-surface Vickers hardness.

Group	Description	Mean \pm S.D.
1	10%Op/5d/SS*	390.3 \pm 7.45
2	10%Op/10d/SS	397.7 \pm 5.79
3	10%NW/5d/SS**	389.8 \pm 4.66
4	10%NW/10d/SS	398.4 \pm 4.30
5	16%NW/5d/SS	385.2 \pm 4.34
6	16%NW/10d/SS	376.2 \pm 4.56
7	15%Op/5d/SS	388.5 \pm 8.02
8	15%Op/10d/SS	393.9 \pm 6.90
9	Control/5d/SS	394.6 \pm 4.90
10	Control/10d/SS	396.1 \pm 3.54

* Op/5d/S = Opalescence treatment for 5 days, surface analysis. ** NW = Nite-White.

For IR and FTIR analysis a 0.56 mg powder tablet was obtained from mixing 1.5% tooth powder by weight with 98.5% potassium bromide (KBr) by weight.

This mixture was placed between two stainless steel disks, air was evacuated with a vacuum pump and a pressure of 4500 kg/cm² was applied for a few mins resulting in tablets measuring 15 x 0.2mm. The spectrum of the prepared tablets was first determined by using IR spectrophotometry followed by FTIR spectrophotometry.

The remainder of the powder, 4.5 mg, was used for XRD analysis. The samples were scanned at a rate of 2 θ = 6°-80° at 10°/min. The radiation utilized was CoK α (λ =1.79) at 40 kV and 20 mA.

Results

The mean and standard deviations of the Vickers hardness measurements of the enamel surface are presented in Table 2, the 110 μm sub-surface measurements in Table 3. All groups, including control groups demonstrated a statistically significant difference between surface VH and subsurface VH values within each group ($P \leq 0.01$); all surface measurements being higher than the 110 μm sub-surface values. The results of a one-way ANOVA of the enamel hardness surface measurements between groups are presented in Table 4, the 110 μm sub-surface analysis in Table 5. All groups had statistically lower VH surface values than the control Groups 9 and 10 ($P \leq 0.01$). Furthermore, Group 6 (16%NW/10d/S) had a statistically significantly lower VH surface hardness than

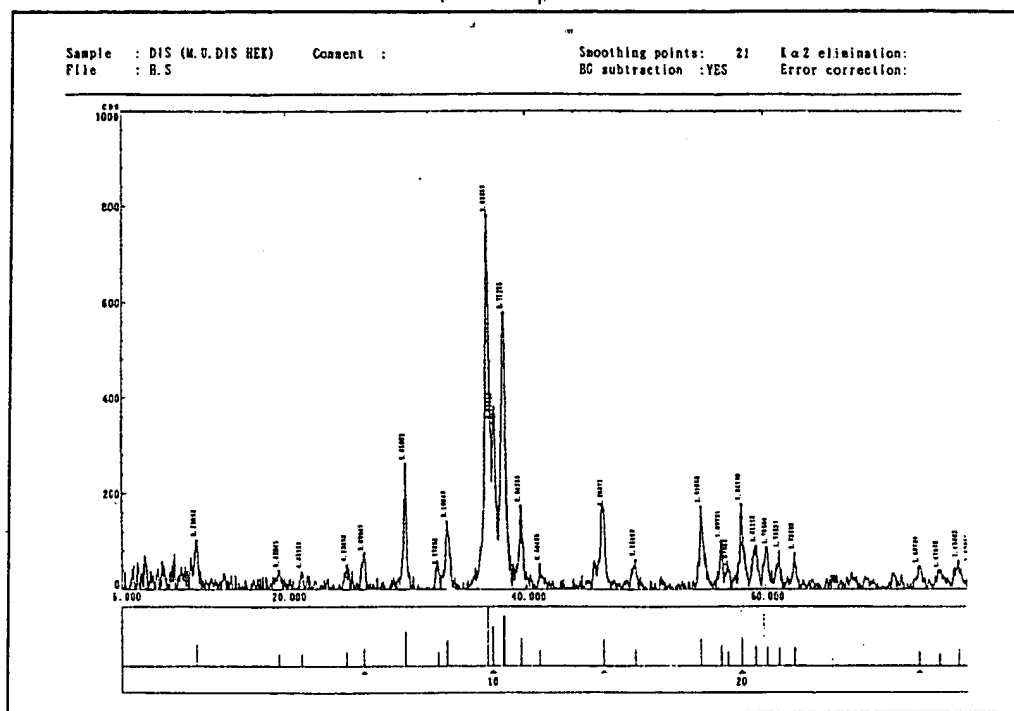


Figure. Representative graph of the XRD analysis. Every peak on this diagram is represented by "d" (d= interplanar distance between atoms) and corresponds to a specific atomic plane. Each peak is specific for a compound. Qualitative analysis identifying the compounds was done using reference cards.

Table 4. Statistically significant differences between the 10 groups in enamel surface Vickers hardness.

Group	1	2	3	4	5	6	7	8	9	10
1 10%Op/5d/S*						X			X	X
2 10%Op/10d/S						X			X	X
3 10%NW/5d/S**						X			X	X
4 10%NW/10d/S						X			X	X
5 16%NW/5d/S									X	X
6 16%NW/10d/S							X	X	X	X
7 15%Op/5d/S									X	X
8 15%Op/10d/S									X	X
9 Control/5d/S										
10 Control/10d/S										

* Op/5d/S = Opalescence treatment for 5 days, sub-surface analysis. ** NW = Nit-White. X Denotes significant differences at 99% confidence level.

Groups 1, 2, 3, 4, 7 and 8. This phenomenon was also observed for the sub-surface VH measurements for Group 6 (16%NW/10d/SS) in that this group had statistically lower values than all other groups ($P \leq 0.01$).

Infrared analysis (IR) - The infrared spectra of Groups 1 and 2 (10%Op/5d and 10%Op/10d) and the control groups, Group 9 and 10 (Ctrl/5d and Ctrl/10d), registered hydroxyapatite [$\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{Cl}, \text{F})$]. The infrared spectra of Groups 3, 7 and 8 (10%NW/5d, 15%Op/5d and 15%Op/10d) recorded the presence of secondary calcium phosphate (CaHPO_4), while the enamel samples of Groups 5 and 6 (16%NW/5d, 16%NW/10d) demonstrated the presence of primary calcium phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2$].

The IR spectra of the control groups (Group 9 and 10) showed peaks at 960 cm^{-1} , 1000 cm^{-1} , and 1100 cm^{-1} corresponding to phosphate. The peaks at 560 cm^{-1} and 590 cm^{-1} corresponded to hydroxyapatite. The IR spectrum of Group 3 produced peaks at 560 cm^{-1} and 590 cm^{-1} which did not conform with the

Table 5. Statistically significant differences between the 10 groups in enamel sub-surface Vickers hardness.

Group	1	2	3	4	5	6	7	8	9	10
1 10%Op/5d/S*				X		X				
2 10%Op/10d/S					X	X				
3 10%NW/5d/S**				X		X				
4 10%NW/10d/S					X	X	X			
5 16%NW/5d/S						X				
6 16%NW/10d/S							X	X	X	X
7 15%Op/5d/S										
8 15%Op/10d/S										
9 Control/5d/S										
10 Control/10d/S										

* Op/5d/S = Opalescence treatment for 5 days, sub-surface analysis. ** NW = Nit-White. X Denotes significant differences at 99% confidence level.

control samples. These peaks were representative of secondary calcium phosphate (CaHPO_4). Tracings from Group 6 demonstrated that the profiles at wavelengths of 560 cm^{-1} and 590 cm^{-1} were almost equal in height. The peaks were indicative of primary calcium phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2$].

Fourier transform infrared spectrophotometry (FTIR) - FTIR spectra of all experimental groups revealed conformity with the control Groups 9 and 10. In spite of the small quantitative differences between control and experimental groups, no significant differences were observed.

X-ray diffraction analysis (XRD) - The XRD analysis of Groups 1 and 2 (10%Op/5d, 10%Op/10d) and control Groups 9 and 10 (Ctrl/5d, Ctrl/10d), recorded the presence of the compound calcium phosphate chloride fluoride hydroxide [$\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{Cl}, \text{F})$], as indicated by card no. JCPDS 25-0166.

The analysis of Groups 3-8 did not conform to the compounds that were recorded for the control groups. For these groups, besides the calcium phosphate chloride fluoride hydroxide [$(\text{PO}_4)_3$]

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(OH,Cl,F)], as indicated by card no. JCPDS 25-0166, also primary calcium orthophosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2$], as indicated by card no: JCPDS 9-0390, was identified.

The figure shows a graph of the XRD analysis. Every peak on this diagram is represented by "d" (d= interplanar distance between atoms) and corresponds to a specific atomic plane. Since each peak is specific for a material, qualitative analysis can be carried out by means of reference cards.

Discussion

Statistically significant differences by means of a one-way ANOVA were established in which the longer duration periods and the higher concentrations of bleaching materials exhibited more of an effect. This was true in particular for Group 6 (16% Nite-White Excell,^d applied for 6 hrs/day for 10 days) for both surface and sub-surface measurements. The statistically significant higher values in surface hardness *versus* sub-surface hardness for all groups including the control groups, can be attributed to the presence of fluorapatite at the surface. This data also demonstrated that although the same concentration was tested for different brands of carbamide peroxide, they did not behave in the same way. Apparently the variations in chemical composition and most likely the vehicle in which the bleach was carried were the reasons for the interaction with enamel or the lack thereof.

The results of IR, FTIR and XRD indicated that a conversion of hydroxyapatite to calcium di-hydrogen phosphate took place over calcium mono-hydrogen phosphate resulting in the loss of Ca^{2+} from enamel. Since the bleaching agents were only applied to the surface and the samples for IR, FTIR and XRD were fabricated from ground enamel, the ratio of enamel in direct contact with the bleaching agent *versus* the total bulk of the sample is extremely small. Therefore the FTIR analysis of all experimental groups only showed a spectrum of unaffected enamel.

The IR analysis, however, demonstrated changes in phase structure, while the XRD indicated changes in chemical composition in enamel, pointing to a certain change in crystalline structure. Interpretation of the final results were gleaned from combining the analysis of the IR, FTIR and XRD.

Solubility products that may be released with their solubility factor are listed below.¹⁶

Ca_3	2.0×10^{-29}
$\text{Ca}_5(\text{PO}_4)_3\text{OH}$	1.6×10^{-58}
CaHPO_4	2.7×10^{-7}
$\text{Ca}(\text{H}_2\text{PO}_4)_2$	1.0×10^{-3}

The groups for which these changes were recorded were Groups 3-8. Only Groups 1 and 2 demonstrated no changes when compared to the control Groups 9 and 10. It is of interest to note that this material is the only ADA-approved bleaching material of the materials tested in this experiment. These findings are in agreement with McCracken *et al.*¹² who also reported a loss of calcium from enamel after 6 hrs of applying a carbamide peroxide formula. Changes in chemical composition of enamel, dentin and cement as well as significant decline in calcium/phosphate ratios have been reported by

Rotstein *et al.*^{13,14} The clinical significance of these findings is difficult to determine since Ca^{2+} and phosphates are present in saliva and can potentially replenish the lost substances. The *in vivo* dynamic interaction of saliva/enamel is a factor that cannot be incorporated in the this *in vitro* experiment. Currently much longer periods of bleaching are prescribed which make the 5- and 10-day applications in this study appear short.

Additional studies should be conducted to determine whether the hydroxyapatite conversion is of a permanent nature and if so, what the long term effect is on the composition and physical properties of enamel. Furthermore, extended periods of application need to be studied to determine whether the long-term effect differs from the 5- and 10-day periods studied.

- Olympus Optical Co., Tokyo, Japan.
- Buehler, Lake Bluff, IL USA.
- Phillips, London, UK.
- ATI Unicam, London, UK.
- Rigaku, Tokyo, Japan.
- Ultradent Products Inc. South Jordan, UT, USA.
- Discus Dental Inc. Beverly Hills, CA, USA.
- Wilson Mechanical Instruments, Bridgeport, CT, USA.
- 3M Dental Products Div., St. Paul, MN, USA.
- Microfix, Tokyo, Japan.

Dr. Cimilli is Assistant Professor, University of Marmara, School of Dentistry, Istanbul, Turkey; Dr. Pameijer is Professor Emeritus, University of Connecticut, Farmington, Connecticut, USA, and a Visiting Professor, Stellenbosch University, Tygerberg, South Africa.

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